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ACKNOWLEDGEMENTS. We thank Dr Rajiv Chikate (Dept. of Chemistry, Abasaheb Garware College, Pune), Dr Prashant Dhakephalkar (Agharkar Research Institute, Pune) and Dr Mugutrao Gaikwad (National Chemical Laboratory, Pune) for their suggestions. Mrs Sharayu Paranjape, Mrs Maithili Jog and Dr Neelima Deshpande helped in the factorial design and statistical analysis, for which we thank them. We are also grateful to Mr Vasudeo Kshirsagar and Dr Sham Diwanay of our Department for their timely suggestions and thank Mr Sane and Mr Kher from the College of Engineering (Metallurgy Dept.), Pune for permitting us to use their mechanical sieve.

Received 8 September 2003; revised accepted 1 January 2004

# Species-specific proteins in closely-related seahorses

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**Non-denatured polyacrylamide gel shows the respective species-specific characteristics on the muscle protein of *Hippocampus kuda* and *H. trimaculatus*. Two proteins of molecular weight 66.8 and 39.8 kDa were found exclusively in *H. kuda*. These constituted about 69.8 and 16.2% respectively of its protein. In *H. trimaculatus*, two other specific proteins with molecular weight of 674.3 and 50.5 kDa were recorded, which constituted 46.0 and 7.5% respectively of its protein. These species-specific proteins are important for species identification, which paves avenues for further characterization and upgrading of the available information on seahorse taxonomy.**

MOST seahorse species were listed as 'vulnerable' in the *Red List of Threatened Animals* during 1996 at the IUCN[1]. Among them, *Hippocampus kuda* has been used to denote almost all the non-spiny seahorses in the Indo-Pacific region. More than 15 names of apparent species could be regarded as merely synonyms of *H. kuda*[2], which suggests that it may incorporate cryptic (morphologically similar, but genetically different) species. Thus, *H. kuda* complex still warrants further research to clarify the relationship among the species it includes. The present study was undertaken with protein markers to identify the species of seahorses *H. kuda* and *H. trimaculatus*[3].

Relatively similar size group of *H. kuda* and *H. trimaculatus* were collected from peninsular Tamil Nadu (India) coast and from each of them 500 mg of tail muscle was dissected out and stored at –30°C. For preparing the extract, the stored samples were homogenized with 400 μl of extracting buffer [Tris base (0.05 M), EDTA (0.01 M), PMSF (0.1 M), β-mercaptoethanol (0.2%), Triton-X 100 (0.1%)] at pH 8 using a glass–glass homogenizer. The homogenates were centrifuged at 4000 *g* for 10 min. From the supernatant the total sarcoplasmic protein was quantified by the method of Lowry *et al.*[4].

The 10% separating and 6.5% stacking non-denatured polyacrylamide gel was prepared according to the standard protocols[5]. For this, 60 μg protein of each sample and 20 μl of molecular weight marker (PMW-H, Genei) with equal volume of sample buffer [1 ml glycerol, 0.1 ml 0.5% bromophenol blue, 1 ml 0.5 M Tris HCl (pH 6.8)] were loaded. The gel was run at constant current of 10 mA for 18 h at 15°C in the presence of tank buffer (0.3% Tris base, 1.4% glycine). The gel was then fixed in 7% acetic acid and stained in a staining solution (0.02% CBB-R 250, 40% methanol, 7% acetic acid). The gel was subsequently de-stained in 7% acetic acid and 40% methanol till clear bands appeared.

The correlation between molecular weight and relative mobility was obtained with the proteins in the molecular weight marker kit (PMW-H). The size of the muscle protein was analysed and documented by a gel documentation software system (Syngene, UK).

The sarcoplasmic proteins which account for 20 to 30% of the total protein in fishes, have a unique property that the separation profile obtained on electrophoresis can be used for unequivocal identification of fish species with reference of authentic sample profile[6]. The electrophoretic pattern obtained for the two seahorse species is shown in Figure 1. The distance travelled by each protein component (expressed as *rf* values) for the two species is given in Table 1. The mobility patterns expressed as densitometry scan graphs for *H. kuda* and *H. trimaculatus* are given in Figure 2 *a* and *b* respectively.

Two proteins with a molecular weight of about 66.8 and 39.8 kDa respectively became specifically apparent in the muscle protein of *H. kuda* (Table 1 and Figure 2). Their concentration was about 69.8 and 16.2% respectively, of the total protein. However, two entirely different proteins with molecular weight of about 674.3 kDa

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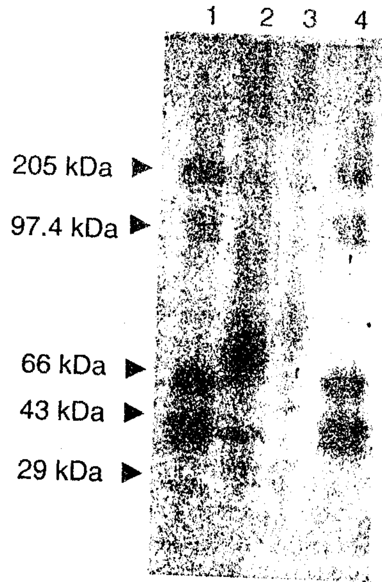


**Figure 1.** Native polyacrylamide gel electrophoresis of muscle extract (60 µg total protein/lane) of seahorse. Lane 1, Molecular weight marker (rabbit muscle myosin 205 kDa; phosphorylase-b: 97.4 kDa; bovine serum albumin: 66 kDa; ovalbumin: 64 kDa; carbonic anhydrase: 29 kDa); Lane 2, *H. kuda* muscle sample; Lane 3, *H. trimaculatus* muscle sample and Lane 4, Molecular weight marker.

43 kDa

and about 50.5 kDa respectively, were distinctly found in the muscle samples of *H. trimaculatus*. Their concentration was 46 and 7.5% respectively, of the total protein.

This result on the specific protein profile provides adequate information to distinguish *H. kuda* from *H. trimaculatus*. Earlier reports indicated that conventional electrophoresis could be adopted effectively for species identification[7]. Usually fish muscle contains three main groups of proteins[8] among which the sarcoplasmic protein (water-soluble) component forms an ideal moiety for electrophoretic method of species identification[6]. In addition, the results of the present study revealed that the native PAGE could form an effective tool to precisely differentiate the related seahorse species. Among methods proposed to find out the details of species origin[9,10], the electrophoretic methods were more reliable for identifying the species, where the usual differences based on morphology of the species are unreliable[11–13]. Considering the lesser morphological differences and similar habitat of both the species, in this study PAGE has revealed distinctive differences in their muscle protein. The value of muscle myogen in phylogenic studies and the intra-specific protein variations as a diagnostic character of fish stock

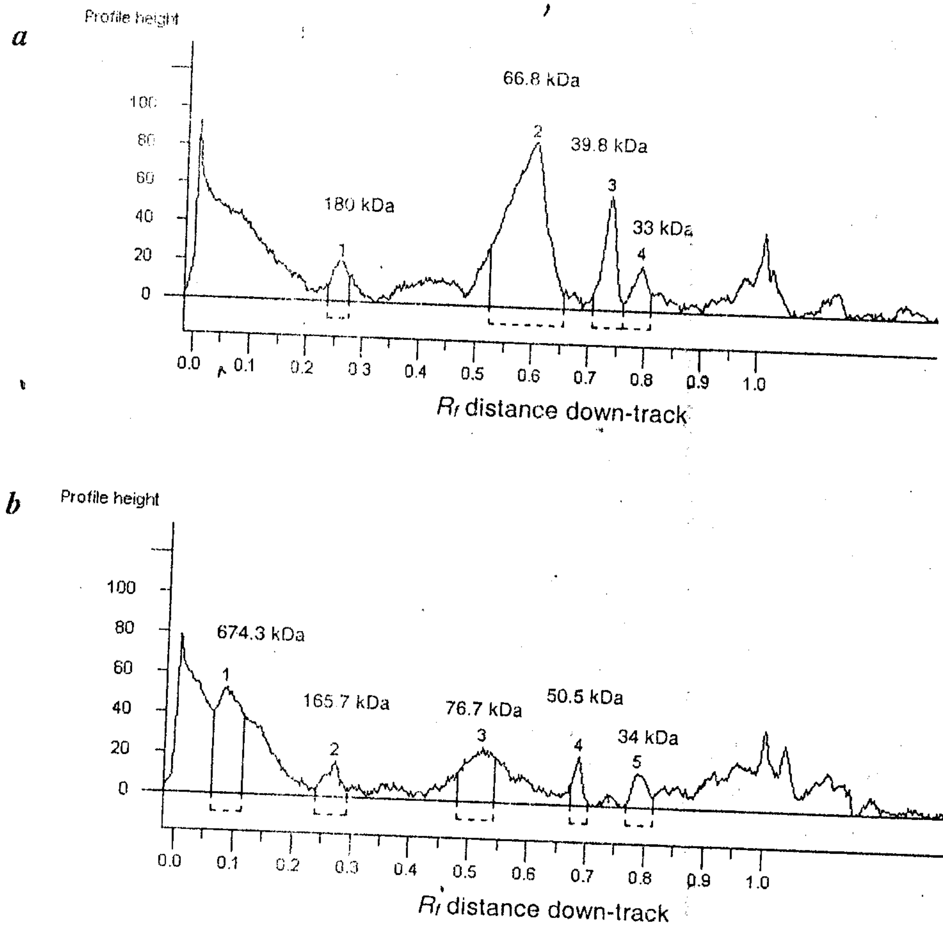


**Figure 2.** Densitometric scan graph of (*a*) *H. kuda* and (*b*) *H. trimaculatus* muscle.

Table 1. Comparison of mobility of muscle proteins in two species of seahorses (bands are numbered from the slowest to the fastest)

| Sample | $R_f$ value from the point of application | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *H. kuda* | 0.2519 | 0.6017 | 0.7373 | 0.7938 | – |
| *H. trimaculatus* | 0.0850 | 0.2720 | 0.5212 | 0.6856 | 0.7875 |

were documented by Tsuyuki *et al.*[14]. The inter-specific, racial and subpopulation studies which were based on meristic and morphometric studies could be thus supplemented by biochemical studies. The *Oncorhynchus* genus could be characterized by relative absence of intra-specific polymorphism, as revealed by the electrophorogram of different tissues[15].

Immunological techniques for the identification of fin-fish and shellfish flesh would have to be restricted because of the impracticality and high cost towards the preparation of antibodies for large number of fish species, in contrast to other sources of meat such as pork and beef. The immunological cross-reactivity of a protein in its native state in various species of fish protein has also been shown[15]. Considering these, the electrophoretic technique can be regarded as a reliable tool for the identification of related seahorse species. Since there is no information on seahorse species, identification of these species-specific proteins is a maiden attempt. These proteins are important for species identification which can be further characterized and used as a tool for upgrading the existing information on seahorse taxonomy.

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ACKNOWLEDGEMENTS. We thank Prof. Mohan Joseph Modayil, Director, CMFRI for facilities and encouragement. We are grateful to Prof. T. J. Pandian, Madurai Kamaraj University, Madurai for reading the manuscript and offering suggestions. We also thank the Ministry of Environment and Forests, Govt. of India for the sponsored research project and research fellowship.

Received 21 July 2003; revised accepted 1 January 2004

# Channel shifting of a highly sinuous meandering river in alluvial plain, Vishwamitri river, Mainland Gujarat

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**River meandering is an inherent characteristic of drainages in an alluvial plain. However, the style and degree of meandering depends on a number of geological factors, including tectonics. Here, we have investigated the Vishwamitri river, a tributary of Dhadhar river flowing through Gujarat alluvial plain. The river follows a slope deviatory course and exhibits a narrow, highly sinuous and deeply incised meandering channel. Several lines of evidence, including satellite and topographic data, stratigraphic and sedimentological data and subsurface structural data have helped in understanding the controls on the channel morphology of the Vishwamitri river. The study has also revealed that the course of the Vishwamitri river has shifted towards east in the last 35 years, in response to neotectonic activity.**

THE channel pattern of a river depends on its planform geometry and the processes operating within its reach[1].

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